Zinc and essential fatty acid status and supplementation in cystic fibrosis patients

S.Van Biervliet

Pediatric Department, UZ Ghent, Ghent, Belgium
stephanie.vanbiervliet@ugent.be

CF:	cystic fibrosis
Zn:	zinc
ALA:	alfalinolenic acid
LA:	linoleic acid
FA:	fatty acid
AA:	arachidonic acid
EPA:	eicosapentaenoic acid
DHGLA:	dihomogammalinoleic acid
DHA:	docosahexaenoic acid
Vit:	vitamin
IAP:	intestinal alkaline phosphatase

Introduction

Cystic fibrosis (CF) is an autosomal recessive disease caused by a mutation in the CF Transmembrane Conductance Regulator (CFTR) located on chromosome 7. CF is a multiorgan disease with pulmonary inflammation and infection as major cause of death. However, nutritional issues play an important role in CF care, since a better survival and slower pulmonary decline was demonstrated in patients with a normal body weight. As 90% of CF patients have exocrine pancreatic insufficiency, pancreatic enzyme replacement and fat – soluble vitamin supplementation is common practice. However, patient with CF might be at risk for other deficiencies which could also influence the clinical course of the disease. However, there are no sufficient research data to support general or selected supplementation. This thesis deals with 2 of these elements: zinc and fatty acids.

Zinc (Zn) is the second most abundant trace element in the human body. Its functions can be divided into catalytic, structural and regulatory. It plays an important role in enzymes, transcription factors, membranes, hormonal receptor sites and so on. Due to its importance in so many cellular functions, the symptoms of Zn deficiency are very general including anorexia, immune dysfunction, growth impairment and essential fatty acid disturbances. These problems are also often encountered in CF patients. Zn is mainly found in meat.

Absorption will be impaired by unabsorbed fatty acids, as demonstrated by Krebs. Formation of unabsorbable soaps by zinc and unabsorbed fatty acids causes a problem in cystic fibrosis since many patients continue to have fat malabsorption despite the correct intake of pancreatic enzyme replacement therapy.

To evaluate the Zn status one can use the serum Zn concentration. However, there are some limitations, since the Zn concentration remains normal for a long period despite insufficient intake. Further on, there is a diurnal variation, with the highest Zn value in the morning. Serum Zn will also decrease in case of inflammation. Further on one can use Zn dependant enzymes, as e.g. alkaline phosphatase, to evaluate the Zn status. Finally the best way to evaluate is a double blind, placebo controlled supplementation study.

Fatty acids (FA) are carbon chains divided in saturated, monounsaturated or polyunsatured, depending on the number of double bounds in the carbon chain. The first double binding from the methyl end on will indicate the family they belong to: omega 3, 6, 7 or 9 fatty acids.

Omega 3 alfalinolenic acid (ALA) and omega 6 linoleic acid (LA) are essential for the human being since they cannot be synthesized by the human body and are needed for correct cellular functioning.

The FAs can be elongated and desaturated into the different FAs of their family.

However, these long chain polyunsaturated FAs can also be present in some food elements.

The functions of FAs are energy source and reserve. They are important for the biomembrane structure. The fluidity of the membrane will be dependent of the amount of long chain polyunsaturated FAs. FAs have also direct influences on the membrane protein functions. Finally, some of them are the precursors of eicosanoids and docosanoids (as thromboxanes, prostaglandins, leukotrienes…) which are important for the initiation and stopping of inflammatory processes. Depending on the balance of the composition of FAs in membranes, there will be more or less pro-inflammatory eicosanoids produced. Arachidonic acid (AA) is the FA producing the most important proinflammatory eicosanoids. The ″3 eicosapentaenoic acid (EPA) and ″6 dihomogammalinoleic acid (DHGLA) are precursors of less pro-inflammatory or even anti-inflammatory eicosanoids.

The FA status can be evaluated by determination of serum FAs. To decrease the impact of recent nutrition the most reliable

parameter is the serum phospholipid FA composition. Total serum FA composition is a much less reliable determination, as compositional differences occur due to recent dietary intake. In case of essential FA deficiency, the human body elongates and desaturates ″9 oleic acid to produce mead acid and its derivatives. The ratio of ″6 AA over ″3 docosahexaenoic acid (DHA) is thought to indicate the inflammatory aspects of the fatty acid profile.

There is a close **interaction between essential FAs and Zn**, which was initially speculated, based upon the similarity of deficiency symptoms: skin lesions, impaired growth, impaired wound healing and delayed sexual maturation. FA abnormalities were described in acrodermatitis enteropathica, an inherited disease of Zn absorption and in transient Zn deficiency. Hamilton described an association between Zn and AA in CF.

Animal studies have until now not been able to elucidate the exact way of interaction.

Cunane et al. postulated a desaturation defect. However, his results were not confirmed by others. Eder et al. proposed an interference with the incorporation of the FA in the phospholipids. Further on, an increased beta-oxidation of LA has been described in Zn deficiency and finally, Zn and LA will improve each others absorption.

Study aims:

The aim of this thesis was to evaluate the Zn and essential FA status in our CF population and evaluate whether supplementation of Zn or essential FAs could be useful for the clinical course of the disease.

Results:

Studies on Zn in CF:

To be able to compare the data of our CF population to local healthy controls, 447 healthy children were studied during a vaccination campaign. Our local control group [1] has a significantly lower serum Zn concentration than described in literature.

The Zn concentration at diagnosis and after one year of therapy was determined in 32 newly diagnosed CF patients [2]. At diagnosis the Zn concentration was 10.7 µmol/L and 12.1 after one year. Compared to the local age matched control group, serum Zn in CF was not different. However, the serum zinc concentration significantly increased after one year of Zn supplementation therapy. Comparing our data on Zn in CF with the data of NHANES II study, including more the 4000 children, 40% of the CF patients at diagnosis and 12.5% after one year of therapy had serum Zn values below the third percentile. These results confirm the data of Krebs et al., observed in young CF patients. Our serum Zn values are comparable to theirs.

The observed association with vitamins A and E is probably caused by the concurrent steatorrhoea.

Since the serum Zn concentration increases with age and therapy, treated CF patients were studied [3]. 101 treated CF patients had a Zn determination. The median Zn concentration was 12.2 µmol/L. The serum Zn from the older patients was not significantly different from our healthy controls. But again 16.5% fall below the NHANES II references. There was an association of serum Zn with serum albumin, which is not surprising since Zn is transported on serum albumin.

Zn interferes also in different ways in the vit A metabolism. It could explain its association with serum vit A levels. Since no association was found with serum vit E, fat malabsorption is probably a less important factor for the explanation of the relation zinc -vit A, in this treated group.

The observed association of serum Zn with the mean forced vital capacity was a surprising observation.

Although speculations on better nutritional status, less inflammation or better immune function can be made, this observation merits a large prospective double blind supplementation study, for further confirmation of the relation of pulmonary function in CF and improved the Zn status.

Serum Zn is as explained the best available parameter for evaluation of Zn status in populations. However, a normal serum Zn concentration does not exclude a Zn deficiency. Therefore intestinal alkaline phosphatase a Zn dependant enzyme was studied. Intestinal alkaline phosphatase (IAP) is an interesting enzyme since it needs Zn or magnesium for its function but also its gene transcription is activated by a Zn containing protein. Finally, this enzyme is very sensitive for peroxidation. Zn deficiency, a condition prone to peroxidative processes, will therefore decrease the enzyme protein content and its activity, resulting in enzyme inactivation.

The brush border enzyme activity of 61 newly diagnosed CF patients was determined [4, 5]. A damaged mucosa was present in 1/3 of the CF population. The controls consisted of children with unexplained failure to thrive. Regardless of their mucosal histology, CF patients have a significant decrease of their IAP activity. On the contrary we found decreased lactase activity in the CF cohort with normal mucosa.

In conclusion, there are arguments for a Zn deficiency in some of the CF patients. As retrospective evaluation showed improved clinical outcomes in Zn supplemented patients, a prospective study is urgently needed.

Studies on essential FAs in CF:

In CF the fatty acid disturbances are known since the sixties [6,7] Different causes have been proposed. First of all, the historical fat-free diet could induce essential FA deficiencies. However, dietary advice has changed dramatically and disturbances in the FA profile are still present. Malabsorption and malnutrition could cause increased losses and beta-oxidation for energy production, resulting in an essential FA deficiency. However, FA disturbances are also present in pancreatic sufficient patients and in well-nourished patients. Due to the presence of double bonds essential FAs are extremely sensitive for oxidation. The more double bonds present, the more vulnerable the FA. An increased oxidative stress has been described in CF induced

by tissue inflammation and infection. A key role could also be attributed to the defective glutathione transport of the CFTR protein. However, improvement of the anti-oxidant status was not able to normalize the fatty acid abnormalities.

Finally phospholipase A2, the key enzyme responsible for FA release from membrane phospholipids, is abnormally regulated in CF. The release of AA by phospholipase A2 is a rate limiting step in the production of pro-inflammatory eicosanoids.

These data are probably linked to the overall pro-inflammatory condition in CF.

The interest in the FA status of CF patients increased since the report by Freedman et al. They demonstrated the same FA disturbances in CF mice and were able to reverse the symptoms using pharmaceutical doses of DHA.

In a cross-sectional study the FA status was determined in 104 CF patients from our CF centre [8]. Eight patients were pancreatic sufficient, 15 had liver disease and 13 had diabetes. They were divided into two groups according to genotype (group A contained patients with two mutations resulting in absence of functional CFTR protein and group B contained patients with at least one mutation resulting in decreased protein function or the mutations with unknown effect on the protein). No differences of age, nutritional status, pulmonary function or serum vit E concentration were seen between the 2 groups. Pancreatic sufficiency was only present in the less severe mutations (group B). Consequently their caloric intake was also significantly lower.

In the ″6 FAs class the differences between the total CF patient group and the healthy controls are: lower plasma phospholipid LA, increased DHGLA and docosapentaenoic acids. In the ″3 fatty acid class lower DHA is observed.

As seen in other essential FA deficiencies, the observed essential FA deficiencies induce an increased Mead acid formation, the elongation-desaturation product of the ″9 FAs. However, the observed FA abnormalities are always less pronounced for less severe mutations (group B).

The only clinical parameter influencing the FA status was the presence of liver disease. The CF patients with liver disease have an even more pronounced decrease of their DHA, even compared to patients of the same genotype.

There was a weak association between serum vit E and plasma phospholipid DHA, probably pointing to increased DHA oxidation, in presence of a suboptimal anti-oxidant status.

Since the observed FA disturbances could negatively influence the clinical outcome of the CF patients by promoting inflammation, an intervention study was performed. DHA is chosen as the FA to be supplemented, since Freedman described normalisation of the symptoms observed in CF mice treated with this FA. Our study was double blind placebo controlled, and was performed in delta F 508 homozygous patients without liver disease [9]. This genotype subgroup was chosen since we wanted only severe mutations to be included. During a full year they had to take either the DHA rich algal oil or sunflower seed oil. Of the 17 patients included, there was one drop out due to Clostridium colitis. There were no relevant clinical differences between the groups at any time point.

The fasting plasma phospholipid concentrations were not different at the start and they displayed the same disturbances as described in the fatty acid genotype study. DHA supplementation, increases significantly its plasma PL levels. The ratio of the omega 6 phospholipid-AA over the omega 3 phospholipid-DHA, pointing to the proinflammatory fatty acid profile of the CF patient, decreased significantly by supplementation. The other important finding was the increased of EPA, caused by retroconversion of DHA to EPA.

Also phospholipid docosapentaenoic acid Mead acids decreased, proving the better DHA status with supplementation. Finally there was a decrease of the ″-6 phospholipid DHGLA and AA, probably induced by the inhibitory effect of DHA on the delta 6 and delta 5 desaturase activity.

From our observations we can conclude that it is possible to shift the FA profile of CF patients to less pro-inflammatory profiles. In contrast to the spectacular results of Freedman in CF mice, no clinical benefits could be observed in this small study group.

Conclusion :

As a general conclusion from our studies we have established normal serum Zn values in our country to be lower than the literature data. Compared to local controls CF patients as a total group, do not have significantly lower serum Zn concentrations as. However there is a subgroup of 40% at diagnosis and 16% of the older CF patients falling below references. The decrease in IAP could also reflect this deficiency.

The factors influencing the FA status are linked to genotype severity and liver disease and should therefore get special attention in future FA studies.

On the question of DHA improving clinical outcomes, no definite answer could be given. Despite the observed the fatty acid shifts, no clinical differences could be observed between our treated and placebo patients.

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